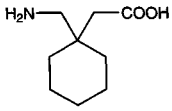
REFERENCE
Gatti,R.; Gotti,R.; Bonazzi,D.; Cavrini,V. A comparative evaluation of three detectors in the HPLC analysis of sodium fusidate, *Farmaco*, **1996**, *51*, 115–119.

Gabapentin



Molecular formula: $C_9H_{17}NO_2$
Molecular weight: 171.24
CAS Registry No.: 60142-96-3
Merck Index: 4343

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL buffer, do not allow to go dry. Condition an Empore C18 SPE membrane by adding 500 μL MeOH, force through three drops, discard MeOH remaining in reservoir, add 500 μL water, force through three drops, discard water remaining in reservoir. Add 200 μL 3 μg/mL IS in buffer to the SPE cartridge, add 200 μL serum and force through at 1 drop/s, add 200 μL buffer, force all liquid through, elute with 500 μL MeOH. Add 100 μL saturated sodium tetraborate solution and 50 μL 5% 2,4,6-trinitrobenzenesulfonic acid in water to the eluate, mix, heat at 50° for 10 min, add 500 μL 250 mM acetic acid, centrifuge at 12500 g for 2 min, add the supernatant to the SPE membrane, force through using a syringe or by centrifuging at 100-120 g for 5 min, wash with 500 μL MeCN:water 20:80, elute with 75 μL MeCN then 125 μL water, mix the eluates, inject a 50 μL aliquot. (Buffer was saturated sodium tetraborate solution diluted with three volumes of water.)

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Ultrasphere C18
Mobile phase: MeCN:water:acetic acid:n-butylamine 52:48:0.1:0.01 (pH should not exceed 4.5) (Connect a 150 × 4.6 37-53 μm silica (Whatman) column between pump and injector.)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 10
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (13)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine epoxide, carbamazepine, chlordiazepoxide, demoxepam, desalkylflurazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, disopyramide, ethosuximide, flurazepam, gentamicin, lidocaine, phenobarbital, phenytoin, primidone, procainamide, quinidine, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
SPE; derivatization; pharmacokinetics

REFERENCE
Lensmeyer,G.L.; Kempf,T.; Gidal,B.E.; Wiebe,D.A. Optimized method for determination of gabapentin in serum by, *Ther.Drug Monit.*, **1995**, *17*, 251–258.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Plasma + 100 μL 2 M perchloric acid, vortex for 10 s, centrifuge at 15000 g for 3 min. Remove a 50 μL aliquot and add it to 200 μL MeOH, add 200 μL buffer, add 50 μL reagent, mix, let stand at room temperature for 5 min, inject a 20 μL aliquot. (Prepare buffer weekly by adjusting the pH of 500 mM boric acid to 9.5 with 1 M NaOH. Prepare reagent weekly by dissolving 50 mg o-phthalaldehyde in 4.5 mL MeOH, add 500 μL buffer, add 50 μL 3-mercaptopropionic acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere octadecyl
Mobile phase: MeCN:MeOH:buffer 30:30:40 (Prepare the buffer by diluting 7.5 mL glacial acetic acid to 400 mL with water, adding 40 mg EDTA, and adjusting the pH to 3.7 with 3 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 11.5
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (Parke-Davis) (15)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: alanine, arginine, aspartic acid, carbamazepine, clobazam, clonazepam, cysteine, felbamate, glutamic acid, glycine, histidine, isoleucine, lamotrigine, leucine, lysine, methionine, oxcarbazepine, phenobarbital, phenylalanine, phenytoin, primidone, proline, remacemide, serine, threonine, tiagabine, tyrosine, valine, valproic acid, vigabatrin

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KEY WORDS
derivatization; plasma

REFERENCE
Forrest,G.; Sills,G.J.; Leach,J.P.; Brodie,M.J. Determination of gabapentin in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 421–425.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL IS solution + 1 mL MeCN, vortex for 5 min, centrifuge for 15 min. Mix 6 μL buffer, 6 μL reagent, and 6 μL supernatant, let stand for 1 min, inject the whole amount. (Prepare IS solution by dissolving 100 mg gamma-phenyl-gamma-aminobutyric acid and 10 mg 1-(aminomethyl)cycloheptane acetic acid in 500 mL MeCN and 500 mL water. Prepare buffer by dissolving 15.5 mg boric acid in 500 mL water and adjusting to pH 9.5 with concentrated NaOH. Prepare reagent by mixing 100 mg o-phthaldialdehyde, 9 mL MeOH, 1 mL buffer, and 100 μL mercaptoethanol.)

HPLC VARIABLES
Column: 250 × 4 5 μm BANsil C18 (ASMT, Enger, Germany)
Mobile phase: Gradient. A was MeCN:MeOH:0.1% pH 2 phosphoric acid 10:10:80. B was MeCN:MeOH 50:50. A:B 90:10 for 1 min, to 30:70 over 25 min, maintain at 30:70 for 3 min, return to initial conditions over 0.1 min, re-equilibrate for 3.9 min.
Column temperature: 40
Flow rate: 1
Injection volume: 18
Detector: F ex 235 em 435

CHROMATOGRAM
Retention time: 27.0
Internal standard: gamma-phenyl-gamma-aminobutyric acid (Marion Merrel Dow) (23.4), 1-(aminomethyl)cycloheptane acetic acid (Gö-3609, Parke Davis) (28.3)
Limit of detection: 100 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: vigabatrin

KEY WORDS
derivatization; serum; degas mobile phase continuously with helium

REFERENCE
Juergens,U.H.; May,T.W.; Rambeck,B. Simultaneous HPLC determination of vigabatrin and gabapentin in serum with automated pre-injection derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1459–1471.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL IS solution + 200 μL 1.2 M perchloric acid, vortex, centrifuge at 3000 rpm for 5 min. Remove the supernatant and add it to 50 μL 5% 2,4,6-trinitrobenzenesulfonic acid in water, add 50 μL 50% NaOH, vortex, let stand at room temperature for 30 min, add 200 μL 6 M HCl, add 100 μL saturated NaCl, add 6 mL cyclohexane, shake for 10 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 200 μL EtOH:mobile phase 10:90, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 5 μm Spherisorb ODSIII
Mobile phase: MeCN:100 mM pH 4 ammonium acetate 54:46
Column temperature: 40
Flow rate: 1
Injection volume: 75
Detector: UV 350

CHROMATOGRAM
Retention time: 5.2
Internal standard: 1-(aminomethyl)heptaneacetic acid (Parke-Davis) (13.3)
Limit of detection: 25 ng/mL

KEY WORDS
derivatization; plasma; dog; pharmacokinetics

REFERENCE
Stevenson,C.M.; Radulovic,L.L.; Bockbrader,H.N.; Fleisher,D. Contrasting nutrient effects on the plasma levels of an amino acid-like antiepileptic agent from jejeunal administration in dogs, *J.Pharm.Sci.*, **1997**, *86*, 953–957.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 50 μL serum or urine (diluted between 1:50 and 1:200) to 1 mL MeOH containing 3.4 μg γ-phenyl-γ-amino-n-butyric acid, vortex for 15 s, centrifuge at 2000 g for 10 min, mix 6 μL of the supernatant with 3 μL reagent, inject an aliquot. (Reagent was 10 mL 30 mg/mL o-phthaldialdehyde and 200 μL 2-mercaptoethanol made up to 50 mL with 400 mM pH 9.5 borate buffer (Ther. Drug Monit. 1991, 13, 251).)

HPLC VARIABLES
Column: 125 × 3 5 μm Superspher 60 RP-Select B (Merck)
Mobile phase: Gradient. A was MeCN. B was 20 mM KH_2PO_4 buffer. A:B from 22:78 to 37:63 in 12 min, from 37:63 to 55:45 in 6 min, from 55:45 to 80:20 in 1.5 min, maintain at 80:20 for 2 min
Column temperature: 35
Flow rate: 0.7
Detector: F ex 230 em 455

CHROMATOGRAM
Retention time: 17.3
Internal standard: γ-phenyl-γ-amino-n-butyric acid (14.8)
Limit of detection: 500 nM

OTHER SUBSTANCES
Extracted: vigabatrin

KEY WORDS
derivatization; serum; urine

REFERENCE
Wad,N.; Krämer,G. Sensitive high-performance liquid chromatographic method with fluorometric detection for the simultaneous determination of gabapentin and vigabatrin in serum and urine, *J.Chromatogr.B*, **1998**, *705*, 154–158.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 10 μL IS in water + 5 drops 2 M perchloric acid, vortex vigorously for a few s, centrifuge at 15000 g for 2 min. Remove the supernatant and add it to 500 μL 1 M sodium bicarbonate, add 50 μL 2 M 2,4,6-trinitrobenzenesulfonic acid in water, adjust pH to 8.5 with 100 mM NaOH, let stand for 30 min, add 2 drops of 25% HCl, add 3 mL toluene, shake for 10 min, centrifuge at 5000 g for 2 min. Remove the upper organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL 200 mM pH 8.5 sodium borate buffer, wash with 1 mL cyclohexane:toluene 90:10 by vortexing for 1 min, inject a 10-50 μL aliquot of the aqueous phase. Urine. 10-100 μL Urine + 10 μL 200 μg/mL IS in water, add 500 μL 1 M sodium bicarbonate, add 50 μL 2 M 2,4,6-trinitrobenzenesulfonic acid in water, adjust pH to 8.5 with 100 mM NaOH, let stand for 30 min, add 2 drops of 25% HCl, add 3 mL toluene, shake for 10 min, centrifuge at 5000 g for 2 min. Remove the upper organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL 200 mM pH 8.5 sodium borate buffer, wash with 1 mL cyclohexane:toluene 90:10 by vortexing for 1 min, inject a 10-50 μL aliquot of the aqueous phase.

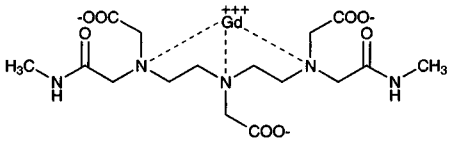
HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:0.5% acetic acid 58:42
Flow rate: 1
Injection volume: 10-50
Detector: UV

CHROMATOGRAM
Retention time: 10.3
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (13.2)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Hengy,H.; Kölle,E.U. Determination of gabapentin in plasma and urine by high-performance liquid chromatography and pre-column labelling for ultraviolet detection, *J.Chromatogr.*, **1985**, *341*, 473–478.

Gadodiamide



Molecular formula: $C_{16}H_{26}GdN_5O_8$
Molecular weight: 573.66
CAS Registry No.: 122795-43-1
Merck Index: 4345

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Filter (Millipore Ultrafree-MC, type PTGC, 10 000 NMWL Filter unit) serum while centrifuging at 4° at 5000 g for 1 h, inject a 10 μL aliquot of the ultrafiltrate. Urine. Centrifuge urine at 4° at 15000 g for 10 min, dilute a 100 μL aliquot of the supernatant with 400 μL water, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2.1 5 μm Supelguard LC-19-DB (Supelco)
Column: 250 × 2.1 5 μm Supelcosil LC-19-DB
Mobile phase: 10 mM Triethylammonium acetate containing 2 mM EDTA, pH adjusted to 6.5-7.0 with 1 M acetic acid or 1 M NaOH
Column temperature: 30
Flow rate: 0.3
Injection volume: 10
Detector: UV 658 following post-column reaction with the reagent pumped at 0.3 mL/min. (Reagent was 100 mM nitric acid containing 0.15 mM Arsenazo III and 10 mM urea, filter (0.45 μm), sonicate, discard after 2 days.)

CHROMATOGRAM

Retention time: 4
Limit of detection: 1.1 μM (urine), 0.3 μM (serum)
Limit of quantitation: 10 μM (urine), 2 μM (serum)

OTHER SUBSTANCES

Extracted: gadopentetate dimeglumine

KEY WORDS

serum; ultrafiltrate; post-column reaction

REFERENCE

Hvattum,E.; Normann,P.T.; Jamieson,G.C.; Lai,J.-J.; Skotland,T. Detection and quantitation of gadolinium chelates in human serum and urine by high-performance liquid chromatography and post-column derivatization of gadolinium with Arsenazo III, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 927–932.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 6 Asahipak ODP-50
Mobile phase: MeCN:pH 6.8 Tris-HCl buffer 1.5:98.5 containing 75 μM n-octylamine
Flow rate: 1.2
Detector: UV 215

CHROMATOGRAM

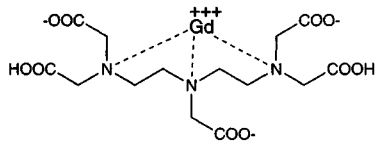
Retention time: 2.4

OTHER SUBSTANCES

Simultaneous: caldiamide, similar chelates of other metals

REFERENCE
Okazaki,O.; Kurata,T.; Yoshioka,N.; Hakusui,H. Pharmacokinetics and stability of caldiamide sodium in rats, *Arzneimittelforschung*, **1996**, *46*, 79–83.

Gadopentetic acid



Molecular formula: $C_{14}H_{20}GdN_3O_{10}$
Molecular weight: 547.58
CAS Registry No.: 80529-93-7, 86050-77-3 (meglumine salt)
Merck Index: 4347

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Filter (Millipore Ultrafree-MC, type PTGC, 10 000 NMWL Filter unit) serum while centrifuging at 4° at 5000 g for 1 h, inject a 10 μL aliquot of the ultrafiltrate. Urine. Centrifuge urine at 4° at 15000 g for 10 min, dilute a 100 μL aliquot of the supernatant with 400 μL water, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Supelguard LC-19-DB (Supelco)
Column: 250 × 2.1 5 μm Supelcosil LC-19-DB
Mobile phase: 10 mM Triethylammonium acetate containing 2 mM EDTA, pH adjusted to 6.5-7.0 with 1 M acetic acid or 1 M NaOH
Column temperature: 30
Flow rate: 0.3
Injection volume: 10
Detector: UV 658 following post-column reaction with the reagent pumped at 0.3 mL/min. (Reagent was 100 mM nitric acid containing 0.15 mM Arsenazo III and 10 mM urea, filter (0.45 μm), sonicate, discard after 2 days.)

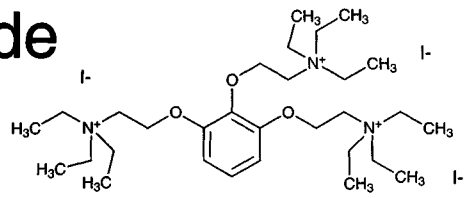
CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Extracted: gadodiamide

KEY WORDS
serum; ultrafiltrate; post-column reaction

REFERENCE
Hvattum,E.; Normann,P.T.; Jamieson,G.C.; Lai,J.-J.; Skotland,T. Detection and quantitation of gadolinium chelates in human serum and urine by high-performance liquid chromatography and post-column derivatization of gadolinium with Arsenazo III, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 927–932.

Gallamine triethiodide



Molecular formula: $C_{30}H_{60}I_3N_3O_3$
Molecular weight: 891.54
CAS Registry No.: 65-29-2
Merck Index: 4361

SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 500 μL MeCN, vortex for 2 min, centrifuge at 15000 g at 4° for 30 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL mobile phase, mix for 2 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak octadecylsilane
Mobile phase: MeCN:buffer 2:98 adjusted to pH 6.0 with 1 M phosphoric acid (Buffer was 5 mM octanesulfonic acid and 5 mM Na_2HPO_4.)
Flow rate: 0.2
Injection volume: 5
Detector: UV 230

CHROMATOGRAM
Retention time: 9.38
Limit of detection: 900 ng/mL

KEY WORDS
plasma

REFERENCE
Shao,M.J.; Fallon,K.D.; Khalil,S.N.; Abouleish,E. Quantitation of gallamine (Flaxedil) in human plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *345*, 184–186.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL 100 μg/mL d-tubocurarine chloride in water + 50 μL 10% sodium tungstate:335 mM sulfuric acid 50:50, vortex for 15 s, centrifuge at 12800 g for 2 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 50 mm long C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:7.5 mM tetrabutylammonium hydrogen sulfate 10:90
Flow rate: 1.8
Injection volume: 30
Detector: UV 229

CHROMATOGRAM
Retention time: 6.35
Internal standard: d-tubocurarine chloride (10.0)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Noninterfering: barbiturates, alcuronium, metocurine, neostigmine, edrophonium

KEY WORDS
serum; rat; pharmacokinetics

REFERENCE
Ramzan,I.M. Determination of the neuromuscular blocking drug gallamine in rat serum using high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *417*, 428–433.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 0.5-400 μg/mL solution in mobile phase.

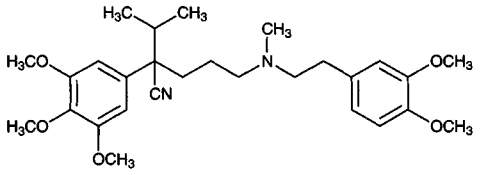
HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil octadecylsilyl
Mobile phase: MeCN:buffer 31:69 containing 100 mM sodium perchlorate (Buffer was 50 mM phosphoric acid adjusted to pH 3 with NaOH.)

Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 12
Limit of detection: 35 ng/mL

REFERENCE
Mourier,P.A. Determination of gallamine and its impurities by reversed-phase ion-pair high-performance liquid chromatography and comparison with thin-layer chromatography, *J.Chromatogr.*, **1989**, *462*, 281–292.

Gallopamil



Molecular formula: $C_{28}H_{40}N_2O_5$
Molecular weight: 484.64
CAS Registry No.: 16662-47-8
Merck Index: 4369

SAMPLE
Matrix: blood
Sample preparation: Condition column A with two 995 μL portions of mobile phase at 3 mL/min and then with 995 μL solution B. Wash the donor channel of the dialyser (Gilson ASTED XL fitted with a Cuprophan cellulose acetate membrane with a molecular mass cut-off of 15000) with 2 mL solution A at 3 mL/min and wash the acceptor channel with 2 mL solution B. Dialyze 370 μL plasma against 9 mL solution B is pumped through the acceptor channel at 1 mL/min. Pass the dialysate through column A, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B. (Solution A was 10 mM pH 3 acetate buffer containing 0.01% Triton X-100 and 50 μg/mL sodium azide. Solution B was 10 mM pH 3 acetate buffer containing 50 μg/mL sodium azide.)

HPLC VARIABLES
Column: A 30 μm Nucleosil CN; B 5 μm LiChrospher 100 RP-18 guard column + 4 μm Superspher 100 RP-18
Mobile phase: MeCN:2-aminoheptane:buffer 25:0.5:75 (Buffer was 10 mM sodium acetate adjusted to pH 3.0 with acetic acid.)
Column temperature: 35
Flow rate: 0.9
Detector: F ex 275 em 310

CHROMATOGRAM
Retention time: 15
Internal standard: gallopamil

OTHER SUBSTANCES
Simultaneous: norverapamil, verapamil

KEY WORDS
dialysate; column-switching; plasma; gallopamil is IS

REFERENCE
Ceccato,A.; Chiap,P.; Hubert,P.; Toussaint,B.; Crommen,J. Automated determination of verapamil and norverapamil in human plasma with on-line coupling of dialysis to high-performance liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, *750*, 351–360.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 60 ng/mL norverapamil in water + 100 μL 2 M KOH, vortex gently for 5 s, add 5 mL hexane:MTBE 80:20, rotate at 45 rpm for 30 min, centrifuge at 1150 g for 5 min, freeze at -80° for 20 min. Remove the organic layer and add it to 100 μL 50 mM pH 3 KH_2PO_4, shake mechanically at high speed for 5 min, centrifuge for 5 min, freeze at -80° for 20 min, discard the organic layer, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 20 mm long 5 μm Supelcosil LC-18-DB
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 50 mM KH_2PO_4 containing 5 mM 1-octanesulfonic acid and 1 mM triethylamine, pH adjusted to 3.0 with phosphoric acid.)
Column temperature: 40
Flow rate: 2
Detector: F ex 205 em 0-56 filter (Kopp) (Emission maximum is 310 nm.)

CHROMATOGRAM

Retention time: 10.1
Internal standard: norverapamil (8.0)
Limit of detection: 0.9 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: hydrochlorothiazide, procainamide, propranolol, quinidine, triamterene

KEY WORDS

plasma; pharmacokinetics

REFERENCE

McLean,A.M.; Babcock-Atkinson,E.; Rein,K.; Ruggirello,D.A.; Gonzalez,M.A.; Noonan,P.K. High-performance liquid chromatographic (HPLC) assay using fluorescence detection for the simultaneous determination of gallopamil and norgallopamil in human plasma, *Pharm.Res.*, **1987**, *4*, 327–331.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.9% NaCl + 120 μL 2 M NaOH + 5 mL n-hexane, vortex for 30 s, mix for 10 min, centrifuge at 2500 g for 20 min, repeat extraction with 4 mL n-hexane. Combine the organic phases and evaporate them to dryness, rinse walls with 1 mL n-hexane, evaporate to dryness, dissolve residue in 50 μL isopropanol, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 Chiralpak AD (Baker)
Column: 250 × 4.6 Chiralpak AD (Baker)
Mobile phase: n-Hexane:isopropanol 90:10 with 0.1% diethylamine
Flow rate: 1
Injection volume: 25
Detector: F ex 223 no emission filter

CHROMATOGRAM

Retention time: 11 (S-(-)), 14 (R-(+))
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: norverapamil
Also analyzed: verapamil

KEY WORDS

plasma; chiral

REFERENCE

Fieger,H.; Blaschke,G. Direct determination of the enantiomeric ratio of verapamil, its major metabolite norverapamil, and gallopamil in plasma by chiral high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 255–260.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve a sample in water, mix a 1 mL aliquot with 100 μL 13.36 μg/m IS, inject an aliquot.

HPLC VARIABLES

Column: 100 × 4.0 5 μm Chiral-AGP (Baker)
Mobile phase: MeCN:pH 6.8 (I = 0.01) ammonium acetate 11:89 (Buffer was adjusted to pH 6.8 with ammonium hydroxide or acetic acid)
Column temperature: 22
Flow rate: 0.9
Injection volume: 20
Detector: UV 225; MS, Finnigan MAT SSQ 710A, interface particle beam, desolvation chamber 45°, nebulizing gas helium, electron impact mode 70 eV, source 250°, filament current 200 μA, electron multiplier 1500 V, m/z 45-400

CHROMATOGRAM

Retention time: 9.39 ((2R)-(+)), 14.26 ((2S)-(-))
Internal standard: procaine hydrochloride (5.09)
Limit of detection: 154 ng/mL ((2R)-(+)), 163 ng/mL ((2S)-(-))

OTHER SUBSTANCES

Also analyzed: verapamil

KEY WORDS

chiral

REFERENCE

Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477–483.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve a sample in water and inject an aliquot.

HPLC VARIABLES

Column: 100 × 4.0 5 μm Chiral-AGP (Baker)
Mobile phase: Gradient. A was MeCN. B was isopropanol. C was pH 6.8 (I = 0.01) ammonium acetate adjusted to pH 6.8 with ammonium hydroxide or acetic acid. A:B:C from 11:1:88 to 7:1:92 over 1.5 min, maintain at 7:1:92 for 35 min, to 11:1:88 over 5 min
Column temperature: 22
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM

Retention time: 11 ((2R)-(+)), 22 ((2S)-(-))

OTHER SUBSTANCES

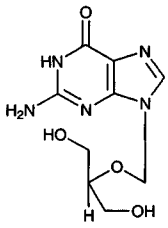
Simultaneous: verapamil

KEY WORDS

chiral

REFERENCE
Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477–483.

Ganciclovir



Molecular formula: $C_9H_{13}N_5O_4$
Molecular weight: 255.23
CAS Registry No.: 82410-32-0, 107910-75-8 (sodium salt)
Merck Index: 4374
Lednicer No.: 5 146

SAMPLE
Matrix: blood
Sample preparation: Add 10 μL 200 μg/mL acyclovir in MeOH to 500 μL plasma, add 1 mL MeCN, vortex briefly, centrifuge at 2000 g for 3 min, add 2 mL chloroform (Caution! Chloroform is a carcinogen!) to the supernatant, vortex. Remove the aqueous supernatant layer, remove traces of the organic solvent under a stream of nitrogen at 80° for 3 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrocart RP8
Mobile phase: MeCN:10 mM pH 5 ammonium acetate buffer 2:98
Flow rate: 1
Injection volume: 30
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: acyclovir (7)
Limit of detection: 3 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Peyrière,H.; Hillaire-Buys,D.; Alric,R. Application of a standardized coextractive cleanup procedure to routine high-performance liquid chromatography assays of teicoplanin and ganciclovir in plasma, *J.Chromatogr.B*, **1998**, *705*, 79–85.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 2 μg 9-methylxanthine + 50 μL 35% perchloric acid, centrifuge at 4° at 2000 g for 15 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM pH 3.50 KH_2PO_4
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: 9-methylxanthine (4.5)
Limit of detection: 0.5 ng

OTHER SUBSTANCES
Noninterfering: acyclovir, allopurinol, azathioprine, caffeine, guanine, guanosine, hypoxanthine, mercaptopurine, oxypurinol, theophylline, uric acid, xanthine

KEY WORDS
plasma

REFERENCE
Boulieu,R.; Bleyzac,N.; Ferry,S. Modified high-performance liquid chromatographic method for the determination of ganciclovir in plasma from patients with severe renal impairment, *J.Chromatogr.*, **1991**, *571*, 331–333.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 35% perchloric acid, centrifuge at 4° at 2000 g for 15 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 3 μm Hypersil ODS
Mobile phase: 20 mM pH 5.25 KH_2PO_4
Flow rate: 1.5
Detector: UV 254

KEY WORDS
plasma

REFERENCE
Boulieu,R.; Bleyzac,N. Stability of ganciclovir in blood samples, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1205–1207.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 4 mL MeCN:10 mM pH 3.2 phosphate buffer, mix, centrifuge at 12000 rpm for 15 min. Remove the supernatant, filter, inject an aliquot. Tissue. Homogenize tissue in MeCN/PBS, centrifuge at 12000 rpm for 15 min. Remove the supernatant, filter, inject an aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:10 mM KH_2PO_4 20:80 (A) or MeOH:10 mM KH_2PO_4 5:95 containing 1 mM tetramethylammonium perchlorate (B)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3 (A), 8 (B)
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; rat; brain; lung; pharmacokinetics

REFERENCE
Brewster,M.E.; Raghavan,K.; Pop,E.; Bodor,N. Enhanced delivery of gangiclovir to the brain through the use of redox targeting, *Antimicrob.Agents Chemother.*, **1994**, *38*, 817–823.

SAMPLE
Matrix: formulations
Sample preparation: Dilute withmobile phase, add hypoxanthine (final hypoxanthine concentration 10 μg/mL), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 6 mM $(NH_4)H_2PO_4$ adjusted to pH 2.5 with 0.33 M phosphoric acid
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 5.96
Internal standard: hypoxanthine (3.94)

KEY WORDS
injections; 10% dextrose; 25% dextrose; stability-indicating

REFERENCE
Johnson,C.E.; Jacobson,P.A.; Chan,E. Stability of gangiclovir sodium and amino acids in parenteral nutrient solutions, *Am.J.Hosp.Pharm.*, **1994**, *51*, 503–508.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Bioblock stirpack homogenizer) 10 mg tissue in 700 μL ice-cold 600 mM perchloric acid at 6000 rpm for 2.5 min, centrifuge at 4° at 2000 g for 15 min, adjust the pH of the supernatant to 7-8 with NaOH. Remove a 200 μL aliquot and add 10 μL alkaline phosphatase (type VII-NT, 10000 glycine U/mg protein, Sigma), heat at 37° for 30 min, evaporate, reconstitute in 30 μL mobile phase, inject a 20 μL aliquot. (The alkaline phosphatase step may be omitted.)

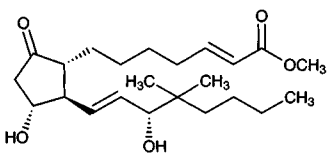
HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM pH 3.5 KH_2PO_4
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.9 pmole/g

KEY WORDS
heart

REFERENCE
Bleyzac,N.; Boulieu,R. High-performance liquid chromatographic determination of ganciclovir nucleotides in human myocardial tissue, *J.Chromatogr.B*, **1994**, *658*, 173–176.

Gemeprost



Molecular formula: $C_{23}H_{38}O_5$
Molecular weight: 394.55
CAS Registry No.: 64318-79-2
Merck Index: 4393
Lednicer No.: 4 11

SAMPLE
Matrix: blood

Sample preparation: Acidify plasma to pH 3.5 with 2 M HCl, pass through a column of Amberlite XAD-2 with a bed volume equal to half the sample volume, wash with water until the eluate is neutral, elute with MeOH (equal to half the original volume), inject an aliquot.

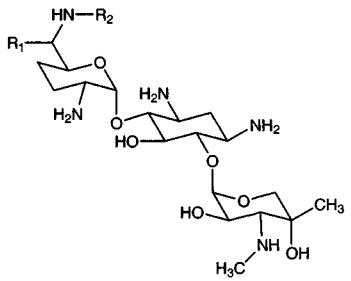
HPLC VARIABLES
Column: 200 × 10 25-40 μm Polygosil 60 C18 glass column
Mobile phase: MeOH:water 83:17
Detector: Radioactivity

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Dimov,V.; Gréen,K.; Bygdeman,M.; Christensen,N.J. Metabolism of 16, 16-dimethyl-*trans*-delta^2-prostaglandin E_1 methyl ester (ONO-802) following intravenous and vaginal administration to pregnant women, *Drug Metab.Dispos.*, **1986**, *14*, 494–502.

Gentamicin

Molecular formula: $C_{19}H_{39}N_5O_7$
Molecular weight: 449.55
CAS Registry No.: 1403-66-3, 1405-41-0 (sulfate)
Merck Index: 4398



Gentamicin C_1 $R_1 = R_2 = CH_3$

Gentamicin C_2 $R_1 = CH_3$, $R_2 = H$

Gentamicin C_{1a} $R_1 = R_2 = H$

SAMPLE
Matrix: blood
Sample preparation: Prepare a column of 150 mg dry silicic acid in a Pasteur pipette plugged with glass wool (10 mm height) and treat with 1 mL water. 500 μL Serum + 1.5 mL water, add to the column, rinse the tube with 1 mL water, add this water to the column, discard the eluate, add 500 μL reagent, let stand for 30 s, elute, discard the eluate, elute with 1.5 mL MeOH. Vortex the eluate, centrifuge, protect from light, inject a 20 μL aliquot. (Elution was performed under positive pressure from a rubber bulb. Reagent was prepared by dissolving 1 g of boric acid in 38 mL water, adjust pH to 10.4 with 450 g/L KOH, add 2 mL 100 mg/mL o-phthalaldehyde in MeOH, add 400 μL 2-mercaptoethanol. Prepare fresh each week.)

HPLC VARIABLES
Guard column: 23 × 3.9 μBondapak C18/Porasil B
Column: 300 × 3.9 10 μm μBondapak
Mobile phase: MeOH:buffer 21:79 (Buffer was 1% triethylamine adjusted to pH 6.2 ± 0.1 with phosphoric acid.)
Flow rate: 2
Injection volume: 25
Detector: F ex 260 em 418

CHROMATOGRAM
Retention time: 4.8 (C1), 6.8 (C1a), 8.6 (C2)
Limit of detection: 80 ng/mL (C1a, C2), 20 ng/mL (C1)

KEY WORDS
serum; derivatization; SPE; pharmacokinetics

REFERENCE
Rumble,R.H.; Roberts,M.S. High-performance liquid chromatographic assay of the major components of gentamicin in serum, *J.Chromatogr.*, **1987**, *419*, 408–413.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 20 μL water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 15 s, centrifuge at 2000 g for 5 min. Filter (0.45 μm, Millex-HV4) the supernatant and add 300 μL of the filtrate to 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN. Heat at 80° for 2 h, cool rapidly to room temperature, filter (0.45 μm, Millex-HV4), inject a 50 μL aliquot of the filtrate. (Buffer was prepared by dissolving 3.81 g disodium tetraborate decahydrate in water, adjusting pH to 10 with NaOH, and making up to 100 mL with water.)

HPLC VARIABLES
Guard column: 33 × 4.6 5 μm C18 (Perkin-Elmer)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 70:30:0.1
Flow rate: 2.2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 11.0
Internal standard: gentamicin C1a

OTHER SUBSTANCES
Extracted: netilmicin

KEY WORDS
serum; guinea pig; human; derivatization; gentamicin C1a is IS

REFERENCE
Dionisotti,S.; Bamonte,F.; Gamba,M.; Ongini,E. High-performance liquid chromatographic determination of netilmicin in guinea-pig and human serum by fluorodinitrobenzene derivatization with spectrophotometric detection, *J.Chromatogr.*, **1988**, *434*, 169–176.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 20 μL water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 15 s, centrifuge at 2000 g for 5 min. Filter (0.45 μm, Millex-HV4) the supernatant and add 300 μL of the filtrate to 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN. Heat at 80° for 2 h, cool rapidly to room temperature, filter (0.45 μm, Millex-HV4), inject a 50 μL aliquot of the filtrate. (Buffer was prepared by dissolving 3.81 g disodium tetraborate decahydrate in water, adjusting pH to 10 with NaOH, and making up to 100 mL with water.)

HPLC VARIABLES
Guard column: 33 × 4.6 5 μm C18 (Perkin-Elmer)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 70:30:0.1
Flow rate: 2.2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 11.0
Internal standard: gentamicin C1a

OTHER SUBSTANCES
Extracted: netilmicin

KEY WORDS
serum; guinea pig; human; derivatization; gentamicin C1a is IS

REFERENCE
Riley,C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1988**, *45*, 2079–2091.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma +20 μL water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 20 s, centrifuge at 2000 g for 5 min. Filter (Millex-HV4) the supernatant. Heat 200 μL filtrate and 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN at 80° for 1 h, cool, inject a 50 μL aliquot. (Buffer was 3.81 g disodium tetraborate decahydrate in water, adjust pH to 10 with NaOH, make up to 100 mL with water.)

HPLC VARIABLES
Guard column: 25 × 4 10 μm LiChroCART RP 18
Column: 250 × 4 5 μm LiChrosorb RP 18
Mobile phase: MeCN:water 70:30 containing 1 mL/L acetic acid
Flow rate: 2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 10.0 (gentamicin C1a)
Internal standard: gentamicin C1a

OTHER SUBSTANCES
Extracted: isepamicin
Noninterfering: ampicillin, aspirin, captopril, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cephalosporins, chlorpromazine, diazepam, heparin, propranolol, sulfamethoxazole, sulpiride, trimethoprim, verapamil

KEY WORDS
plasma; guinea pig; human; derivatization; gentamicin C1a is IS

REFERENCE
Dionisotti,S.; Bamonte,F.; Scaglione,F.; Ongini,E. Simple measurement of isepamicin, a new aminoglycoside antibiotic, in guinea pig and human plasma, using high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1991**, *13*, 73–78.

SAMPLE
Matrix: blood, broth
Sample preparation: Condition a 3 mL 100 mg Isolute CBA-bonded (carboxypropyl) silica SPE cartridge (Jones Chromatography) with 1 mL MeOH and 1 mL 20 mM pH 7.4 phosphate buffer. Add 1 mL plasma or broth to the SPE cartridge, wash with 2 mL 20 mM pH 7.4 phosphate buffer, wash with 4 mL 200 mM pH 9.0 borate buffer, dry with 30 mL air, elute with 1 mL MeCN:200 mM pH 10.5 borate buffer 50:50, force out all the liquid with air. 1 mL Eluate + 200 μL 800 mM boric acid + 200 μL 2.5 mM 9-fluorenylmethyl chloroformate in MeCN, let stand at room temperature for 15 min, add 25 μL 100 mM glycine, let stand for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 3 μm ODS Hypersil
Mobile phase: MeCN:water 87:13
Flow rate: 1
Injection volume: 50
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 18 (C_{1a}), 20 (C_2), 22 (C_{2a}), 24 (C_1)

Limit of detection: 10-50 ng/mL

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Stead,D.A.; Richards,R.M.E. Sensitive fluorimetric determination of gentamicin sulfate in biological matrices using solid-phase extraction, pre-column derivatization with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 295–302.

SAMPLE
Matrix: blood, dialysate, urine
Sample preparation: Plasma. Condition a 3 mL Baker cyanopropylsilane CN SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer. 1 mL Plasma + 100 μL 100 μg/mL dibekacin in water, vortex for 15 s, add 1 mL buffer, vortex for 15 s, centrifuge at 3100 g at 4° for 7 min, add to SPE cartridge, wash with 500 μL water, wash with 250 μL mobile phase, elute to dryness. Elute with 250 μL mobile phase, inject an aliquot of the eluate. Urine, dialysate. Dilute 1:100 with water, add 100 μL 100 μg/mL dibekacin per 1 mL of sample, mix well, inject a 100 μL aliquot. (Buffer was 0.94 g sodium hexanesulfonate in 300 mL water, add 500 μL glacial acetic acid, dilute to 500 mL with water.)

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Hypersil C18
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 15:85 (Buffer was 3.48 g sodium hexanesulfonate + 28.4 g sodium sulfate in 2 L water, acidify to pH 3.4 with 2 mL glacial acetic acid.)
Column temperature: 25
Flow rate: 1.1
Injection volume: 100
Detector: F ex 338 em 418 (bandpass filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.05 mm i.d. knitted PTFE reaction coil at 25° to the detector (Derivatizing reagent was 0.4 g o-phthalaldehyde in 3 mL MeOH added to 390 mL buffer, add 2 mL β-mercaptoethanol, make up to 500 mL with water, store at 4°. Buffer was 1 M pH 10.4 borate from equal volumes of 1 M KOH and boric acid.)

CHROMATOGRAM
Retention time: 11, 17, 17.5, 22
Internal standard: dibekacin

OTHER SUBSTANCES
Simultaneous: kanamycin, isepamicin, tobramycin, netilmicin

KEY WORDS
post-column reaction; SPE; plasma

REFERENCE
Maloney,J.A.; Awni,W.M. High-performance liquid chromatographic determination of isepamicin in plasma, urine and dialysate, *J.Chromatogr.*, **1990**, *526*, 487–496.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.037

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 2 mg/mL solution in 20 mM pH 9.0 borate buffer, remove a 5 mL aliquot and add it to 15 mL 150 mM 2,4-dinitrofluorobenzene in MeOH (prepare fresh daily), heat at 100° for 45 min, cool, make up to 250 mL with mobile phase, discard the upper aqueous phase, inject a 20 μL aliquot of the lower organic phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb SI-100
Mobile phase: Chloroform:THF:water 65:17.5:0.2
Flow rate: 1
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 8, 12, 16

KEY WORDS
normal phase; derivatization

REFERENCE
Tsuji,K.; Goetz,J.F.; VanMeter,W.; Gusciora,K.A. Normal-phase high-performance liquid chromatographic determination of neomycin sulfate derivatized with 1-fluoro-2,4-dinitrobenzene, *J.Chromatogr.*, **1979**, *175*, 141–152.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Weigh out amount equivalent to 100 mg gentamicin, dissolve in 100 mL water. Remove a 20 mL aliquot and add it to 10 mL reagent, make up to 50 mL with MeOH, heat at 90° for 15 min, cool for 5 min, inject a 20 μL aliquot. Injections. 500 μL of a 5% injection + 19.5 mL water + 10 mL reagent, heat at 90° for 15 min, cool for 5 min, inject a 20 μL aliquot. (Reagent was 400 mg o-phthalaldehyde in 4 mL MeOH, add 38 mL buffer, add 0.8 mL thioglycolic acid, adjust the pH to 10.4 with 45% KOH. Buffer was 6.18 g boric acid in 200 mL water, adjust pH to 10.4 with 45% KOH, make up to 250 mL with water.)

HPLC VARIABLES
Guard column: 45 × 4.6 Vydac reversed phase
Column: 150 × 4.6 Ultrasphere ODS

Mobile phase: Gradient. A was MeOH:water:acetic acid 700:250:50 containing 5 g/L sodium heptanesulfonate. B was MeOH. A:B 100:0 for 2 min, to 75:25 over 3 min, maintain at 75:25.
Flow rate: 1.5
Injection volume: 20
Detector: UV 330

CHROMATOGRAM
Retention time: 5.07 (C1), 11.06 (C1a), 12.67 (C2a), 13.77 (C2)

KEY WORDS
injections; derivatization

REFERENCE
Albracht,J.H.; de Wit,M.S. Analysis of gentamicin in raw material and in pharmaceutical preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *389*, 306–311.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a 100-200 μg/mL solution in water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 Carbopac PA-1 anion-exchange (Dionex)
Column: 250 × 4 Carbopac PA-1 anion-exchange (Dionex)
Mobile phase: Gradient. Water:10 mM NaOH from 70:30 to 50:50 over 15 min, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 20
Detector: E, Dionex PED-1 pulsed amperometric detector, gold working electrode, amperometry mode, E1 0.10 V, t1 300 ms, E2 0.60 V t2 120 ms, E3 -0.80 V t3 300 ms following post-column reaction. The effluent from the column was mixed with 500 mM NaOH pumped at 0.5 mL/min and flowed through a mixing coil (Dionex RDM) to the detector.

CHROMATOGRAM
Retention time: 5.91 (C1a), 6.66 (C2), 8.04 (C2a), 10.05 (C1)
Limit of detection: 20 ng

OTHER SUBSTANCES
Noninterfering: cefazolin, clindamycin, cloxacillin, kanamycin, neomycin, penicillin G, tobramycin

KEY WORDS
post-column reaction; injections

REFERENCE
Kaine,L.A.; Wolnik,K.A. Forensic investigation of gentamicin sulfates by anion-exchange ion chromatography with pulsed electrochemical detection, *J.Chromatogr.A*, **1994**, *674*, 255–261.

SAMPLE
Matrix: formulations
Sample preparation: Mix 2 g cream with 3 mL n-butanol, add 5 mL 2% sulfuric acid, mix thoroughly. Separate lower aqueous layer and re-extract the organic layer with another portion of sulfuric acid. Combine the aqueous layers and make up to 100 mL with water. Filter a portion of the extract through a 0.45 μm Nylon 66 syringe filter. Dilute the filtrate with water, inject a 10 μL. aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Metachem Inertsil C8
Mobile phase: 200 mM Sodium sulfate containing0.3 mM sodium 1-heptanesulfonate and 0.1% acetic acid
Flow rate: 1
Injection volume: 10

Detector: F ex 340 em 440 following post-column reaction. The column effluent mixed with reagent pumped at 1.0 mL/min and this mixture flowed through a 9 m × 0.25 mm I.D. stainless steel coil to the detector. (Reagent was 800 mg o-phthalaldehyde and 1 mL mercaptoethanol in 10 mL MeOH diluted to 1 L with 2.5% boric acid and adjusted to pH 10 with 2.5% KOH.)

CHROMATOGRAM
Retention time: 22.0
Limit of detection: 10 ng

OTHER SUBSTANCES
Also analyzed: paromomycin

KEY WORDS
cream; post-column reaction

REFERENCE
Pick,J.; Olson,L.L.; Ellis,W.Y.; Lim,P. Development and validation of a method to extract and quantitate paromomycin and gentamicin from an Aquaphilic cream formulation, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 131–137.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 150 μL sample to 5 mL, inject an aliquot.

HPLC VARIABLES
Guard column: C18 precolumn filter
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeCN:200 mM KH_2PO_4 30:70 adjusted to pH 6.5
Flow rate: 2
Detector: UV 260

CHROMATOGRAM
Retention time: 7.67

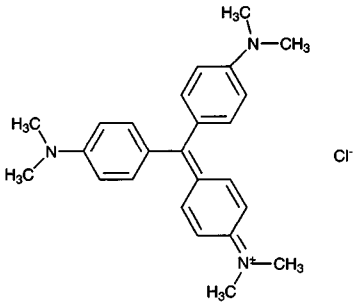
OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating; ophthalmic solutions

REFERENCE
McBride,H.A.; Martinez,D.R.; Trang,J.M.; Lander,R.D.; Helms,H.A. Stability of gentamicin sulfate and tobramycin sulfate in extemporaneously prepared ophthalmic solutions at 8 degrees C, *Am.J.Hosp.Pharm.*, **1991**, *48*, 507–509.

Gentian violet

Molecular formula: $C_{25}H_{30}ClN_3$
Molecular weight: 407.99
CAS Registry No.: 548-62-9
Merck Index: 4401



SAMPLE

Matrix: feed

Sample preparation: Prepare a Sephadex column by slurrying 5 g Sephadex LH-20 with 100 mL MeOH:water 90:10 in a beaker, let stand for 4 h, add to a 300 × 19 column, wash out beaker with MeOH:water 90:10 and add this to the column. Grind feed to pass 1 mm sieve and mix for at least 24 h on a revolving mixer. 10 g Ground feed + 50 mL MeOH:1 M HCl (99:1), shake vigorously on a mechanical shaker for 20 min, centrifuge at 2000 rpm for 15 min, decant, repeat extraction. Combine the extracts, mix well, evaporate an aliquot containing 5 μg gentian violet to dryness under reduced pressure at 48-50°. Reconstitute the residue in 2 mL MeOH:water 90:10, add to the Sephadex column, rinse the flask three times with 2 mL portions and once with a 6 mL portion of MeOH:water 90:10, add the rinses to the column, discard the eluates, elute with 6-6.5 mL MeOH:water 90:10, wash the column tip with 1 mL MeOH:water 90:10, evaporate the eluate to 500 μL under a stream of nitrogen at 48-50°, add 2 mL EtOH, evaporate to dryness under a stream of nitrogen at 48-50°, repeat process, reconstitute the residue in 1 mL MeOH, inject a 3 μL aliquot.

HPLC VARIABLES

Guard column: pellicular C18 (Alltech)
Column: 150 × 3.9 4 μm Nova-Pak RP-C18
Mobile phase: MeOH:buffer 85:15 (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.75
Injection volume: 3
Detector: UV 588

CHROMATOGRAM

Retention time: 4.4
Limit of detection: 3 ng

KEY WORDS

SPE

REFERENCE

Martinez,E.E.; Shimoda,W. Modified liquid chromatographic method for determination of gentian violet in animal feed, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 742–745.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm CN (Alltech)
Mobile phase: MeOH:100 mM sodium acetate 60:40 containing 50 mg/L disodium EDTA, pH adjusted to 4.5 with aldehyde free glacial acetic acid
Flow rate: 0.8
Injection volume: 5-25
Detector: E, Bioanalytical Systems LC-4B, glassy carbon working electrode +1.000 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 11.2
Limit of quantitation: 0.54 ng

OTHER SUBSTANCES
Simultaneous: metabolites, leucogentian violet
Interfering: methylene blue

REFERENCE
Roybal,J.E.; Munns,R.K.; Hurlbut,J.A.; Shimoda,W. High-performance liquid chromatography of gentian violet, its demethylated metabolites, leucogentian violet and methylene blue with electrochemical detection, *J.Chromatogr.*, **1989**, *467*, 259–266.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 2.1 5 μm Ultracarb C18 (Phenomenex)
Mobile phase: MeCN:100 mM pH 4.5 ammonium acetate 80:20
Flow rate: 0.4
Injection volume: 100
Detector: MS, Hewlett-Packard Model 5989A, source 250°, solvation chamber 45°, particle beam nebulizer helium 40-45°, scan m/z 80-500

OTHER SUBSTANCES
Simultaneous: brilliant green, leucogentian violet, leucomalachite green, malachite green, pentamethyl gentian violet, tetramethyl gentian violet

REFERENCE
Turnipseed,S.B.; Roybal,J.E.; Rupp,H.S.; Hurlbut,J.A.; Long,A.R. Particle beam liquid chromatography-mass spectrometry of triphenylmethane dyes: application to confirmation of malachite green in incurred catfish tissue, *J.Chromatogr.B*, **1995**, *670*, 55–62.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL neutral alumina SPE cartridge (J.T. Baker) and a 2.8 mL 500 mg Bond Elut PRS SPE cartridge with 5 mL MeCN. Place the alumina SPE cartridge on the top of the PRS SPE cartridge using a Bond Elut adapter. Mix 3 mL 250 mg/mL hydroxylamine hydrochloride in water with 5 mL 50 mM p-toluene sulfonic acid, 20 mL 100 mM ammonium acetate adjusted to pH 4.5 with glacial acetic acid, and 20 g fish tissue. Homogenize at 20000 rpm for 1 min (Ultra-Turrax T25 Tissuemizer, Tekmar, USA). Add 90 mL MeCN to the sample and homogenize for 10 s. Shake vigorously by hand for 1 min. Add 20 g basic alumina (Brockman activity I), shake for 1 min. Centrifuge, decant the supernatant. Add 30 mL MeCN to the residue, extract, combine the supernatants. Add 100 mL water, 50 mL dichloromethane, and 20 mL diethylene glycol to the supernatants, shake vigorously, separate the bottom layer. Again add 50 mL dichloromethane, shake for 1 min, combine the separated dichloromethane layers. Concentrate to 2-3 mL under reduced pressure at 65°. Add 2 mL dichloromethane and 5 mL MeCN then add the mixtures to the SPE cartridges. Rinse the flask twice with 5 mL portions of MeCN and add the rinses to the SPE cartridges. Wash with 5 mL MeCN to waste. Remove the alumina cartridge. Wash the PRS cartridge with 2 mL water and with 1 mL MeCN:100 mM ammonium acetate buffer 50:50 adjusted to pH 4.5 with glacial acetic acid. Elute with 2 mL MeCN:100 mM ammonium acetate buffer 50:50 adjusted to pH 4.5 with glacial acetic acid and collect in a tube containing 500 μL 2.5 mg/mL hydroxylamine hydrochloride in water. Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.0 pellicular C18
Column: 150 × 4.6 5 μm SynChropak SCD-100 (SynChrom)
Mobile phase: MeCN:buffer 55:45 (Buffer was 400 mg ammonium acetate and 1 mL triethylamine in 400 mL water, adjusted to pH 3.0 with glacial acetic acid, and made up to 450 mL with water.)
Flow rate: 2.0

Injection volume: 100
Detector: E, ESA Coulochem Model, oxidative electrochemical cell (EC) +900 mV; UV 588; F ex 265 em 360

CHROMATOGRAM
Retention time: 7.9
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, malachite green

KEY WORDS
SPE; catfish; trout

REFERENCE
Rushing,L.G.; Hansen,E.B.,Jr. Confirmation of malachite green, gentian violet and their leuco analogs in catfish and trout tissue by high-performance liquid chromatography utilizing electrochemistry with ultraviolet-visible diode array detection and fluorescence detection, *J.Chromatogr.B*, **1997**, *700*, 223–231.

SAMPLE
Matrix: tissue
Sample preparation: Prepare an alumina column by adding 5 g alumina (Alcoa type F-20, 80-200 mesh, activated chromatographic grade) to a 75 × 16 column. Condition a Bond Elut disposable LRC carboxylic acid SPE cartridge with 10 mL MeCN. 25 g Ground tissue + 100 mL MeCN:buffer 80:20, shake vigorously for 1 min, let stand for 30 min, shake for 1 min, protect from light, let stand overnight, centrifuge at 2000 rpm for 15 min, filter (paper) the supernatant, add 100 mL MeCN:buffer 80:20 to the residue, shake vigorously for 15 s, protect from light, let stand for 1 h, centrifuge, filter (paper). Combine the filtrates, add 25 mL water, add 2 mL diethylene glycol, extract with 100 mL dichloromethane. Evaporate the dichloromethane extract just to dryness, add 5 mL MeCN to the residue, add to the alumina column, rinse flask three times with 5 mL portions of MeCN, add the rinses to the column, elute with 10 mL MeCN, call all the eluates and evaporate them just to dryness. Reconstitute the residue in 25 mL dichloromethane, add 15 mL citrate buffer, shake vigorously for 30 s, remove the organic layer, extract the aqueous layer with two 25 mL portions of dichloromethane. Combine the organic layers and evaporate them just to dryness. Reconstitute with 10 mL MeCN, add a 5 mL aliquot to the SPE cartridge, wash with 5 mL MeCN, discard all eluates, elute with two 2 mL portions of acidic MeOH, evaporate eluate under a stream of nitrogen at 40° to 1 mL, inject a 20 μL aliquot. (Citrate buffer (pH 6-7) was prepared fresh just before use from 1 part 1 M HCl and 2 parts saturated sodium citrate. Acidic MeOH was 95 mL, 5 mL water and 2 mL concentrated HCl, dilute a 1 mL aliquot of this solution to 100 mL with MeOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm CN (Alltech)
Mobile phase: MeCN:buffer 50:50 (Buffer was 100 mM sodium acetate adjusted to pH 4.5 with acetic acid containing 50 mg/L EDTA.)
Flow rate: 1
Injection volume: 20
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode 1.000 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 16
Limit of detection: <10 PPB

OTHER SUBSTANCES
Extracted: metabolites, leucogentian violet

KEY WORDS
chicken; liver; muscle; SPE; protect from light

REFERENCE

Roybal,J.E.; Munns,R.K.; Hurlbut,J.A.; Shimoda,W. Determination of gentian violet, its demethylated metabolites, and leucogentian violet in chicken tissue by liquid chromatography with electrochemical detection, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 940–command.946.

SAMPLE

Matrix: tissue

Sample preparation: Condition at 6 mL 1 g neutral alumina SPE cartridge (J.T. baker) with 5 mL MeCN. Condition a 2.8 mL 0.5 g Bond Elut PRS SPE cartridge with 5 mL MeCN. 10 g Muscle tissue + 3 mL 250 mg/mL hydroxylamine hydrochloride in water + 5 mL 50 mM p-toluenesulfonic acid + 10 mL 100 mM pH 4.5 ammonium acetate buffer, homogenize (Tekmar Ultra-Turrax T25 tissuemizer) at 20000 rpm for 1 min, add 90 mL MeCN, homogenize for 10 s, shake vigorously by hand for 1 min, add 20 g basic alumina (Brockman activity I, Fisher Scientific), shake vigorously for 1 min, centrifuge, decant, add 30 mL MeCN to the residue, extract, centrifuge, decant. Combine the supernatants and add 100 mL water, add 50 mL dichloromethane, add 2 mL diethylene glycol, shake vigorously by hand for 1 min, let stand for 45 min, remove the lower organic layer, add 50 mL dichloromethane, shake for 1 min, let stand for 5 min, remove the lower organic layers. Combine the organic layers and evaporate them to 2-5 mL under reduced pressure at 65°, add 2 mL dichloromethane, add 5 mL MeCN, add the mixture to the alumina SPE cartridge on top of the PRS SPE cartridge, rinse the flask with two 5 mL portions of MeCN, add the rinses to the SPE cartridges, wash the SPE cartridges with 5 mL MeCN, wash with 1 mL solvent, elute with 1.5 mL solvent, add 500 μL water to the eluate, inject a 100 μL aliquot. (Solvent was MeCN:100 mM ammonium acetate 50:50, adjusted to pH 4.5 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 20 × 2 pellicular CN

Column: 250 × 4.6 5 μm LC-CN (Supelco)

Mobile phase: MeCN:buffer 60:40 (Prepare buffer by dissolving 3.85 g ammonium acetate in 380 mL water, adjust to pH 4.5 with glacial acetic acid, make up to 400 mL with water.)

Flow rate: 1

Injection volume: 100

Detector: UV 588 following post-column oxidation. The column effluent passed through a 20 × 2 column packed with lead(IV) oxide to the detector (Mallinckrodt)

CHROMATOGRAM

Retention time: 12.6

Limit of quantitation: 1 ng/g

OTHER SUBSTANCES

Extracted: leucogentian violet

Simultaneous: methyl violet

KEY WORDS

fish; muscle; catfish; SPE; post-column reaction

REFERENCE

Rushing,L.G.; Webb,S.F.; Thompson,H.C.,Jr. Determination of leucogentian violet and gentian violet in catfish tissue by high-performance liquid chromatography with visible detection, *J.Chromatogr.B*, **1995**, *674*, 125–131.

SAMPLE

Matrix: tissue

Sample preparation: Condition at 6 mL 1 g neutral alumina SPE cartridge (J.T. baker) with 5 mL MeCN. Condition a 2.8 mL 0.5 g Bond Elut PRS SPE cartridge with 5 mL MeCN. 20 g Muscle tissue + 3 mL 250 mg/mL hydroxylamine hydrochloride in water + 5 mL 50 mM p-toluenesulfonic acid in water + 20 mL 100 mM pH 4.5 ammonium acetate buffer, homogenize (Tekmar Ultra-Turrax T25 tissuemizer) at 20000 rpm for 1 min, add 90 mL MeCN, homogenize for 10 s, shake vigorously by hand for 1 min, add 20 g basic alumina (Brockman activity I, Fisher Scientific), shake vigorously for 1 min, centrifuge, decant, add 30 mL MeCN to the residue, extract, centrifuge, decant. Combine the supernatants and add 100 mL water, add 50 mL dichloromethane, add 2 mL diethylene glycol, shake vigorously by hand for 1 min, let stand

for 45 min, remove the lower organic layer, add 50 mL dichloromethane, shake for 1 min, let stand for 5 min, remove the lower organic layer, repeat the extraction with 50 mL dichloromethane. Combine the organic layers and evaporate them to 2-5 mL under reduced pressure at 65°, add 2 mL dichloromethane, add 5 mL MeCN, add the mixture to the alumina SPE cartridge on top of the PRS SPE cartridge, rinse the flask with two 5 mL portions of MeCN, add the rinses to the SPE cartridges, wash the SPE cartridges with 5 mL MeCN. Discard the alumina cartridge, wash the PRS cartridge with 2 mL water, wash with 1 mL solvent, elute with 2 mL solvent, add 500 μL 2.5 mg/mL hydroxylamine hydrochloride in water to the eluate, inject a 100 μL aliquot. (Solvent was MeCN:100 mM ammonium acetate 50:50, adjusted to pH 4.5 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 20 × 2 pellicular C18
Column: 150 × 4.6 5 μm SynChropak SCD-100 (SynChrom)
Mobile phase: MeCN:buffer 55:45 (Prepare buffer by dissolving 400 mg ammonium acetate and 1 mL triethylamine in 400 mL water, adjust to pH 3.6 with glacial acetic acid, make up to 450 mL with water.)
Flow rate: 2
Injection volume: 100
Detector: UV 588 following post-column oxidation. The column effluent passed through a 20 × 2 column packed with lead(IV) oxide (Mallinckrodt) to the detector.

CHROMATOGRAM

Retention time: 8.2
Limit of detection: 1.8 ng/g
Limit of quantitation: 3 ng/g

OTHER SUBSTANCES

Extracted: leucogentian violet, leucomalachite green, malachite green, methyl violet

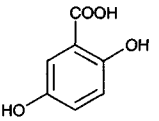
KEY WORDS

fish; muscle; catfish; trout; SPE; post-column reaction

REFERENCE

Rushing,L.G.; Thompson,H.C.,Jr. Simultaneous determination of malachite green, gentian violet and their leuco metabolites in catfish and trout tissue by high-performance liquid chromatography with visible detection, *J.Chromatogr.B*, **1997**, *688*, 325–330.

Gentisic acid



Molecular formula: $C_7H_6O_4$
Molecular weight: 154.12
CAS Registry No.: 490-79-9, 4955-90-2 (sodium salt)
Merck Index: 4404

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

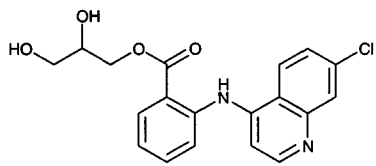
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazide, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Glafenine



Molecular formula: $C_{19}H_{17}ClN_2O_4$
Molecular weight: 372.81
CAS Registry No.: 3820-67-5
Merck Index: 4443

SAMPLE

Matrix: blood, formulations

Sample preparation: Blood. Add 3 mL MeOH:aqueous ammonia solution 99.5:0.5 to 500 μL serum, shake vigorously, centrifuge at 300 rpm for 15 min, evaporate the supernatant to dry-

ness under a stream of nitrogen, dissolve the residue in 200 μL MeOH, filter (0.2 μm Fluorepore), inject an aliquot. Tablets. Dissolve 200 mg (2 tablets) in 500 mL 100 mM HCl, stir at 100 rpm, filter, dilute 2 mL of the filtrate to 100 mL with 100 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm μBondapak C18
Mobile phase: MeOH:water:H_3PO_4 40:60:0.25, pH adjusted to 3.5 with 100 mM NaOH
Flow rate: 1.5
Injection volume: 10
Detector: UV 360

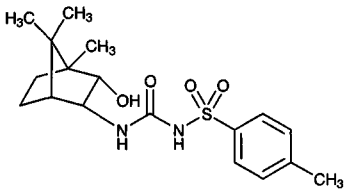
CHROMATOGRAM
Retention time: 7.65
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rat; serum; tablets

REFERENCE
Hassan,S.S.M.; Elnemma,E.M.; Abbas,A.B. Determination of glafenine in dosage forms and serum by thin layer densitometry and high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 215–221.

Glibornuride



Molecular formula: $C_{16}H_{26}N_2O_4S$
Molecular weight: 366.48
CAS Registry No.: 26944-48-9
Merck Index: 4447
Lednicer No.: 2 117

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM
Retention time: 5.92
Limit of detection: <120 ng/mL

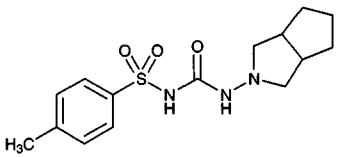
KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Gliclazide



Molecular formula: $C_{15}H_{21}N_3O_3S$
Molecular weight: 323.42
CAS Registry No.: 21187-98-4
Merck Index: 4448

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

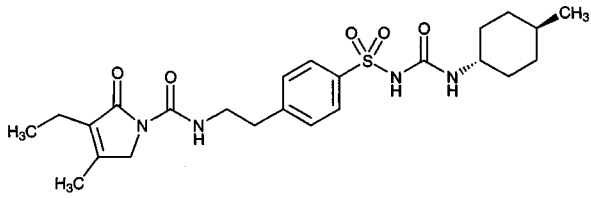
CHROMATOGRAM
Retention time: 20.5

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Glimepiride

Molecular formula: $C_{24}H_{34}N_4O_5S$
Molecular weight: 490.62
CAS Registry No.: 93479-97-1
Merck Index: 4449



SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 40 μL 10 μg/mL IS1 in MeOH containing 10 μg/mL IS2 + 1 mL 50 mM pH 1 HCl/KCl buffer + 5 mL diethyl ether, shake for 20 min, centrifuge at 2500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL reagent, heat at 100° for 20 min, evaporate to dryness under a stream of nitrogen at 60°, reconstitute with 200 μL initial mobile phase, inject a 100 μL aliquot. (Prepare reagent by dissolving 30 μL 2,4-dinitrofluorobenzene in 10 mL n-butyl acetate.)

HPLC VARIABLES
Column: 125 × 4.6 5 μm Spherisorb ODS
Mobile phase: Gradient. MeCN:50 mM perchloric acid 40:60 for 6 min, to 58:42 (step gradient), maintain at 58:42 for 8 min, re-equilibrate at initial conditions for 2 min.
Flow rate: 2
Injection volume: 100
Detector: UV 350

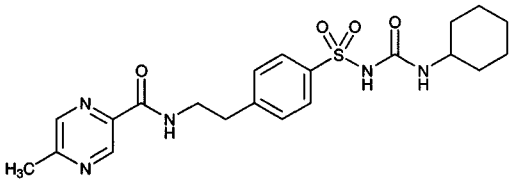
CHROMATOGRAM
Retention time: 11.3
Internal standard: IS1 (1-[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenylsulfonyl]-3-(4-ethylcyclohexyl)urea) (13.4), IS2 (1-[4-[2-(5-chloro-2-methoxyphenyl-1-carboxamido)ethyl]phenylsulfonyl]-3-(4-hydroxycyclohexyl)urea) (3.4)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: glibornuride, glyburide, tolbutamide

KEY WORDS
derivatization; serum; silanize glassware with dichlorodimethylsilane; pharmacokinetics

REFERENCE
Lehr,K.H.; Damm,P. Simultaneous determination of the sulphonylurea glimepiride and its metabolites in human serum and urine by high-performance liquid chromatography after pre-column derivatization, *J.Chromatogr.*, **1990**, *526*, 497–505.

Glipizide



Molecular formula: $C_{21}H_{27}N_5O_4S$
Molecular weight: 445.54
CAS Registry No.: 29094-61-9
Merck Index: 4451
Lednicer No.: 2 117

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

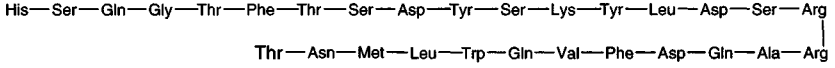
CHROMATOGRAM
Retention time: 17.603

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Glucagon

Molecular formula: $C_{153}H_{225}N_{43}O_{49}S$
Molecular weight: 3482.82
CAS Registry No.: 9007-92-5, 16941-32-5
Merck Index: 4455



SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in dilute HCl, inject a 2.5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Polygosil C18 (Macherey Nagel) (Condition column as follows. Elute with a linear gradient from MeOH to toluene, pump 10 mL 0.1 g/mL chlorodimethyloctylsilane in toluene through at 0.2 mL/min at 48°, flush (at 1 mL/min) with a linear gradient of MeOH, MeOH:water:trifluoroacetic acid 50:50:0.1, MeOH, and MeOH:chloroform 50:50, flush overnight at 0.2 mL/min with MeOH with a gradient.)
Mobile phase: MeOH:water:trifluoroacetic acid 64:36:0.1
Column temperature: 30
Flow rate: 1.2
Injection volume: 2.5
Detector: UV 205

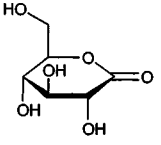
CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Interfering: secretin

KEY WORDS
pig

REFERENCE
Olieman,C.; Sedlick,E.; Voskamp,D. In situ silylation of an octadecylsilyl-silica stationary phase applied to the analysis of peptides, such as secretin and glucagon, *J.Chromatogr.*, **1981**, *207*, 421–424.

Gluconolactone



Molecular formula: $C_6H_{10}O_6$
Molecular weight: 178.14
CAS Registry No.: 90-80-2
Merck Index: 4465

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.1 PRP-X300 (Hamilton)
Mobile phase: 50 mM pH 4.5 NaH_2PO_4
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 5.3

OTHER SUBSTANCES
Simultaneous: gluconic acid, dextrose

REFERENCE
Baxter Scientific Products Catalog, 1990-1, p. 123.

SAMPLE
Matrix: urine
Sample preparation: Lyophilize a 5 mL aliquot of urine at -50° for 18 h, reconstitute the residue in 5 mL DMF, centrifuge at 3000 g for 10 min. 1 mL Supernatant + 300 μL phenylisocyanate, heat at 100° for 1 h, cool, add 500 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm ODS 224 RP18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV

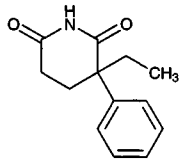
CHROMATOGRAM
Retention time: 9
Limit of detection: 0.4 ng

OTHER SUBSTANCES
Extracted: galactonolactone, galactitol
Simultaneous: dextrose, galactose, allose, myoinositol, sorbitol, mannitol

KEY WORDS
derivatization

REFERENCE
Rakotomanga,S.; Baillet,A.; Pellerin,F.; Baylocq-Ferrier,D. Simultaneous determination of gluconolactone, galactonolactone and galactitol in urine by reversed-phase liquid chromatography: application to galactosemia, *J.Chromatogr.*, **1991**, *570*, 277–284.

Glutethimide



Molecular formula: $C_{13}H_{15}NO_2$
Molecular weight: 217.27
CAS Registry No.: 77-21-4
Merck Index: 4485
Lednicer No.: 1 257

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.

Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 24.3
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

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KEY WORDS
serum

REFERENCE
Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 11.91
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

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KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 259

CHROMATOGRAM

Retention time: 3.83

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool 1 mL microsomal incubation to 0°, add 3 mL ethyl acetate, shake in a reciprocal shaker for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute with running buffer, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm RP 8 (Merck)
Column: 250 × 4 5 μm Superspher RP 8
Mobile phase: MeCN:10 mM pH 6.5 tetrabutylammonium hydrogen sulfate 30:70
Flow rate: 0.7
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
comparison with CE; rat; liver

REFERENCE
Weinz,C.; Blaschke,G.; Schiebel,H.-M. Investigation of the stereoselective in vitro biotransformation of glutethimide by high-performance liquid chromatography and capillary electrophoresis, *J.Chromatogr.B*, **1997**, *690*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 1 5 μm LiChrosorb RP18
Mobile phase: EtOH:water 20:80 containing 20 mM α-cyclodextrin and 0.5 mM tri-O-methyl-α-cyclodextrin
Column temperature: 20
Flow rate: 0.04
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.6, k' 2.9 (enantiomers)

OTHER SUBSTANCES
Extracted: mephobarbital

KEY WORDS
chiral

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS

Mobile phase: MeCN:water:glacial acetic acid 4:84:12 containing 4.84 g/L Trizma, pH 2.3
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.87
Internal standard: 8-chlorotheophylline (5.29)

OTHER SUBSTANCES
Simultaneous: acetaminophen, caffeine, cefazolin, cimetidine, ergotamine, heparin, methamphetamine, propranolol, salicylic acid, sulfamethoxazole, theobromine, theophylline, tobutamide, trimethoprim
Noninterfering: amitriptyline, amobarbital, ampicillin, butabarbital, butalbital, celbenine, chlordiazepoxide, chlorpromazine, clorazepate, desipramine, diazepam, doxepin, ethchlorvynol, fluphenazine, hydroxyzine, ibuprofen, imipramine, isoniazid, lidocaine, mephobarbital, mesoridazine, methaqualone, methyluric acid, naprotyline, nordiazepam, nortriptyline, oxazepam, pentobarbital, perphenazine, phenelzine, phenmetrazine, phenobarbital, phenylbutazone, phenytoin, prednisolone, prednisone, procainamide, prochlorperazine, promazine, promethazine, propoxyphene, protriptyline, pyrilamine, secobarbital, thioridazine, thiothixene, timolol, trazodone, triazolam, trifluoperazine

REFERENCE
Osterloh,J.; Yu,S. Simultaneous ion-pair and partition liquid chromatography of acetaminophen, theophylline and salicylate with application to 500 toxicologic specimens, *Clin.Chim.Acta*, **1988**, *175*, 239–248.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: Hexane:EtOH 60:40
Column temperature: 23
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 8.73 (R-(+)), 16.20 (S-(-))

OTHER SUBSTANCES
Simultaneous: 4-hydroxyglutethimide

KEY WORDS
chiral

REFERENCE
Aboul-Enein,H.Y.; Islam,M.R. Isocratic high-performance liquid chromatographic resolution of glutethimide enantiomers and their 4-hydroxyglutethimide metabolites using cellulose tribenzoate chiral stationary phase, *J.Chromatogr.Sci.*, **1990**, *28*, 307–310.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.644

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sul-

faethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

Flow rate: 0.6

Injection volume: 25

Detector: UV 229

CHROMATOGRAM

Retention time: 6.60 (A), 6.24 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: MeOH
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 10 (R-(+)), 16 (S-(-))

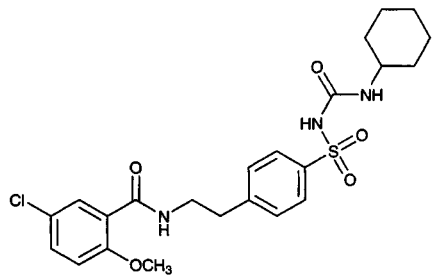
OTHER SUBSTANCES
Simultaneous: metabolites, 5-hydroxyglutethimide

KEY WORDS
chiral

REFERENCE
Weinz,C.; Blaschke,G.; Schiebel,H.-M. Investigation of the stereoselective in vitro biotransformation of glutethimide by high-performance liquid chromatography and capillary electrophoresis, *J.Chromatogr.B*, **1997**, *690*, 233–242.

Glyburide

Molecular formula: $C_{23}H_{28}ClN_3O_5S$
Molecular weight: 494.01
CAS Registry No.: 10238-21-8
Merck Index: 4486
Lednicer No.: 2 139



SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229